REMARKS

This is in response to the Advisory Action mailed on January 31, 2005. The application continues to include claims 1-9, 11-28, and 34-43.

In the Advisory Action, the Examiner entered the Amendment in response to the Final Office Action advising Applicant that Applicant's reply had overcome the rejections under 35 U.S.C. § 112, second paragraph, but had not overcome the rejections under 35 U.S.C. § 103. Applicant's attorney thanks the Examiner for the consideration that the Examiner has given to Applicant's remarks in the last response.

The Examiner also noted in the Advisory Action that the article by John A. Kiernan was not found attached to the Amendment. Applicant's records show that the article was sent in with the Amendment. In any event, a copy of that article is enclosed with this Amendment for the Examiner's review.

At this time, it is believed that a review of the invention would be worthwhile. Applicant is claiming a tissue and a method of crosslinking tissue using a component that acts as a "bridge". To act as a "bridge", the bridging molecule cannot react with other bridging molecules. A bridging molecule cannot react with other bridging molecules for the simple fact, that if it did, then it would not be a "bridge". It would be a randomly reacted molecular mass. The bridging molecule also cannot react randomly with the underlying tissue, but only at selected sites which are termed in the claims as "linkers". The bridges have functional groups which react with the linkers and therefore act as "bridges" between linker sites. The claims also state that the linkers and the bridges are chemically different. All of the above are elements of the claims as they presently stand.

In the Advisory Action, the Examiner is still maintaining the rejection under 35 U.S.C. § 103 based on the Ogle

U.S. Patent No. 5,958,669 in view of the Yang et al. U.S. Patent No. 5,935,168. Applicant believes that this combination does not meet the elements of the claims in the instant application. In the combination of references that the Examiner cites, and if Applicant's attorney understands the Examiner correctly, the Examiner suggests that the diamine would be a linker and the glutaraldehyde would act as a bridge. This combination however would not meet the elements of the claims in the instant application.

The reason that this combination would not meet the elements of the claims is that the glutaraldehyde cannot function as a bridge since the aldehyde groups of the glutaraldehyde would not only react with the diamines (linkers in the Examiner's combination), but also with any other primary nitrogens in the tissue. It is well noted that tissue contains amine groups, and that the functional groups in the glutaraldehyde readily react with such amine groups. (See Figure 4 of Kiernan).

Furthermore, glutaraldehyde readily self-polymerizes as the article by Kiernan indicates. This is another reason why the glutaraldehyde cannot act as a bridge. If glutaraldehyde were to be used as the "bridging" material, the reaction would include self-polymerization of the glutaraldehyde along with reaction with the diamines acting as linkers and reaction with any amines found in the underlying tissue.

The Examiner's own reference, Ogle et al., acknowledges self-polymerization. It discusses the use of a semi-permeable membrane to provide a selected molecular weight distribution of oligomers of glutaraldehyde which are spontaneously formed (self-polymerized). The thrust of the Ogle et al. patent is to provide oligomers of a selected molecular weight to control crosslinking (fixing the tissue). The manner in which this is done is to use a membrane of a selected pore size that retains large oligomers (which are not desirable for crosslinking) on one side of the

membrane while oligomers of a desirable size pass through and are used for crosslinking the tissue. The reason for the separation step is due to spontaneous self-polymerization.

The present application also recognizes that glutaraldehyde cannot be used as a bridge and does not list glutaraldehyde as a bridge but as a linking agent with bridging molecules having diamino groups (Page 27, Lines 17-19) (See also Page 19, Lines 14-18 for discussion of aldehyde linkers and diamino bridges).

The relevancy of the Examiner's comments are not understood regarding that Ogle et al. disclose that the concentration of glutaraldehyde can be lowered to form a higher quantity of monomers and small oligomers (Col. 6, Lines 26-29), and that dilution produces more monomers and small oligomers resulting from dilution affecting the equilibrium between monomers and oligomers in polymerizing that results in oligomers. The Examiner further states: "If polymerizing resulted from covalent bonding due to covalent reacting between aldehyde groups as asserted by Applicants, concentration will not affect whether monomers or oligomers are formed since in a reaction that involves covalent bonding an equilibrium between reacted and non-reacted components does not exist."

Applicant's attorney does not understand the Examiner's above comments. First, even in a covalent bonding situation, an equilibrium exists between reacted and non-reacted components. There are components (whether monomers or oligomers) that are ready to react, but will not react simply due to physical constraints that come to exist in a polymerization reaction. Second, even when a covalent reaction occurs, de-polymerization occurs and an equilibrium exists between unreacted monomers, oligomers and polymers. See attached Chapter on "Polymerization Conditions and Polymer Reactions, E. Degradation of Polymers,

Textbook of Polymer Science", Second Edition, Fred W. Billmeyer, Jr., 1971.

Applicant's attorney also does not understand the Examiner's comments in the Advisory Action that:

> "The specification discloses nothing that will prevent self-polymerizing of glutaraldehyde. Applicants argue that glutaraldehyde cannot form a bridge. However, glutaraldehyde can clearly form a bridge when two amine groups are present. See Figure 2 of Yang et al."

The Examiner is correct in stating that applicant's specification discloses nothing that will prevent self-polymerization of glutaraldehyde but the relevancy of this non-disclosure is not understood.

Applicant has asserted that glutaraldehyde self-polymerizes. Glutaraldehyde cannot act as a bridge material. Glutaraldehyde will self-polymerize and react with the underlying tissue. (See Kiernan's Figure 3B and associated discussion relating to aldehyde side-chains and Figure 4) Applicant's claims clearly state that the bridges are generally non-reactive with other bridges. Therefore, glutaraldehyde cannot be a bridge material.

The Examiner's reference to Figure 2 of Yang et al. is also not understood. All Yang et al. are showing in Figure 2 is the reaction of interest in their patent application. This does not mean that other reactions do not exist. Figure 2 of Yang et al. is a reaction equation which shows the reactivity of glutaraldehyde with amine groups to provide crosslinking. This does not mean that the functional aldehyde groups do not engage in other reactions. As Kiernan points out in his article, polymized glutaraldehyde has a number of side-chain aldehyde groups and there is ready reaction with protein nitrogens (tissue nitrogens), but even after that reaction with protein nitrogens, there are many left-over aldehyde groups (not bound to anything)

which cause problems in non-specific binding (other reactions) and which must be blocked. None of these reactions are shown in Figure 2 of Yang et al., but still exist. Therefore, Figure 2 of Yang et al. is not dispositive of all the reactions glutaraldehyde actually undergoes.

In view of the above, it is respectfully requested that the Examiner reconsider the rejection of all the claims and allow the claims.

The Director is authorized to charge any fee deficiency required by this paper or credit any overpayment to Deposit Account No. 23-1123.

Respectfully submitted,

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Formaldehyde, formalin, paraformaldehyde and glutaraldehyde: What they are and what they do.

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Aldehydes are the most commonly used fixatives. They serve to stabilize the fine structural details of cells and tissues prior to examination by light or electron microscopy. Research workers, technicians, pathologists and others who regularly use aldehyde fixatives frequently do not appreciate the nature and properties of these compounds or the reasons for choosing to fix a specimen in formaldehyde, glutaraldehyde or a mixture of the two. Misconceptions are widespread also about formalin and paraformaldehyde, the commercial products from which formaldehyde-containing solutions are made.

Properties of formaldehyde and its polymers

Formaldehyde is a gas. Its small molecules (HCHO, of which the -CHO is the aldehyde group) dissolve rapidly in water, with which they combine chemically to form methylene hydrate, $HO\text{-}CH_2\text{-}OH$. This is the form in which formaldehyde exists in aqueous solutions; its chemical reactivity is the same as that of formaldehyde. Methylene hydrate molecules react with one another, combining to form polymers (Fig. 1). The liquid known as formalin contains 37-40% of formaldehyde and 60-63% of water (by weight), with most of the formaldehyde existing as low polymers (n = 2 to 8 in the formula given in Fig. 1). Higher polymers (n up to 100), which are insoluble, are sold as a white powder, paraformaldehyde.

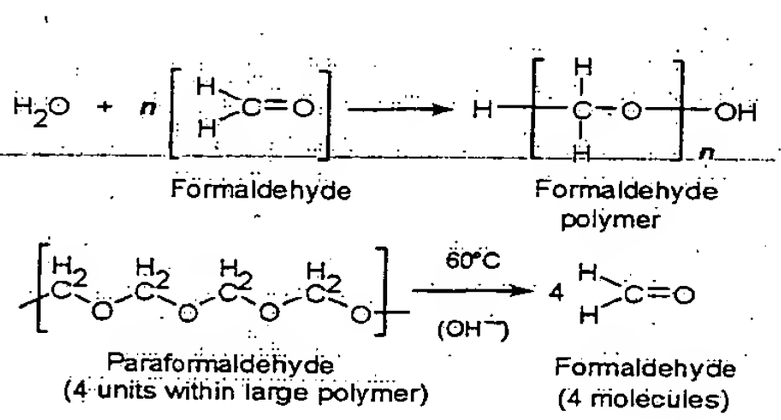

Fig. 1. Formation of formaldehyde polymers (above), and depolymerization of paraformaldehyde (below).

To be useful as a fixative, a solution must contain monomeric formaldehyde (or methylene hydrate, to be pedantic) as its major solute. Dilution with water breaks up the small polymers in formalin. This process is said to take a couple of days if plain water is used, but to be almost instantaneous when formalin is diluted with a buffer solution at physiological pH (Pearse, 1980). Hydrolysis of the polymers is catalyzed by the hydroxide ions present in the slightly alkaline solution (Fig. 1). The big polymer molecules in paraformaldehyde need more energetic treatment. Heating is necessary, as is an added source of hydroxide ions. In one of the earliest paraformaldehyde-derived fixatives (Richardson, 1960) this was sodium sulfite, but the regular practice for at least 35 years has been simply to heat the paraformaldehyde to 60C in water containing the salts used to buffer the solution to pH 7.2 to 7.6.

Formalin contains about 10% methanol, added by the manufacturer because it slows down the polymerization that leads eventually to precipitation of paraformaldehyde. A 4% formaldehyde solution made from formalin therefore contains about 1% methanol. It also contains a small amount of formate ions. These are derived from the Cannizzaro reaction, in which two formaldehyde molecules react together, one being reduced to methanol and the other oxidized to formic acid. Because of this slow reaction, the concentrations of methanol and formate in any formaldehyde solution increase slowly with prolonged storage (Walker, 1964). A solution of formaldehyde prepared from paraformaldehyde, which does not initially contain any methanol, is commonly used in fixatives for electron microscopy and in research applications. Satisfactory ultrastructural preservation is, however, also seen in tissues fixed in buffered formaldehyde generated from formalin (Carson, *et al.*, 1973).

Reaction of formaldehyde with proteins

The aldehyde group can combine with nitrogen and some other atoms of proteins, or with two such atoms if they are very close together, forming a cross-link -CH2- called a methylene bridge. Studies of the chemistry of tanning indicate that the most frequent type of cross-link formed by formaldehyde in collagen is between the nitrogen atom at the end of the side-chain of lysine and the nitrogen atom of a peptide linkage (Fig. 2), and the number of such cross-links increases with time (Gustavson, 1956). The tanning of collagen to make leather is comparable to the hardening of a tissue by a fixative (Hopwood, 1969). The fixative action of formaldehyde is probably due entirely to its reactions with proteins. Initial binding of formaldehyde to protein is largely completed in 24 hours (Helander, 1994) but the formation of methylene bridges proceeds much more slowly. Substances such as carbohydrates, lipids and nucleic acids are trapped in a matrix of insolubilized and cross-linked protein molecules but are not chemically changed by formaldehyde unless fixation is prolonged for several weeks.

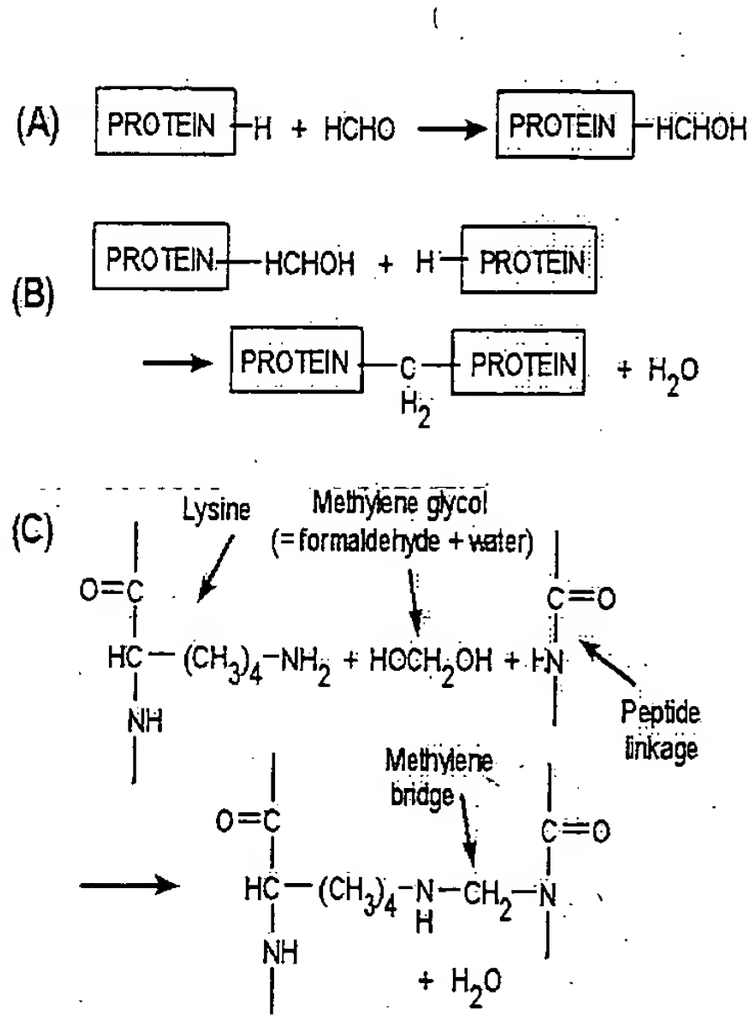

Fig. 2. Reactions involved in fixation by formaldehyde. **(A)** Addition of a formaldehyd molecule to a protein. **(B)** Reaction of bound formaldehyde with another protein molecule to form a methylene cross-link. **(C)** A more detailed depiction of the cross-linking of a lysine side-chain to a peptide nitrogen atom.

Practical considerations relating to formaldehyde

This is the most important bit. Formaldehyde penetrates tissues quickly (small molecules), but its reactions with protein, especially cross-linking, occur slowly. Adequate fixation takes days, especially if the specimen must withstand the osmotic and other stresses of dehydration and infiltration with paraffin. Brief fixation in formaldehyde (ideally delivered by perfusion) can stop or greatly reduce autolysis and confer slight hardening and some resistance (but not much) to liquids that are not iso-osmotic with the tissue. This can greatly improve the structural integrity of cryostat and other frozen sections, especially if followed by infiltration with a cryoprotectant such as sucrose (ideally 60% but more usually 15-30%).

When a specimen is dehydrated after only a few hours in formaldehyde, the largely unfixed cytoplasmic proteins are coarsely coagulated. Nuclear chromatin, which contains DNA and strongly basic proteins, is also coagulated by the solvent, forming a pattern of threads, lumps and granules. This is not unlike the appearance induced by fixatives that contain acetic acid, but it is less satisfactory for identifying cell-types on the basis of nuclear morphology. (After adequate formaldehyde fixation, chromatin displays a remarkably even texture, also of little diagnostic value but possibly closer to the structure of the living nucleus.)

Glutaraldehyde solutions

Before 1962 the only satisfactory fixative for electron microscopy was buffered osmium tetroxide. This preserves cellular structure by combining with lipids, especially in membranes, and by insolubilizing some proteins without coagulation, but it is expensive and toxic, penetrates tissues extremely slowly, and extracts much protein and RNA. With the introduction of glutaraldehyde (Sabatini *et al.*, 1962) electron microscopists had a more rapidly penetrating fixative that thoroughly insolubilized proteins and was cheap enough to deliver by vascular perfusion.

Glutaraldehyde has fairly small molecules, each with two aldehyde groups, separated by a flexible chain of 3 methylene bridges. It is HCO-$(CH_2)_3$-CHO. The potential for cross-linking is obviously much greater than with formaldehyde because it can occur through both the -CHO groups and over variable distances. In aqueous solutions, glutaraldehyde is present largely as polymers of variable size (Monsan *et al.*, 1975). There is a free aldehyde group sticking out of the side of each unit of the polymer molecule (Fig. 3), as well as one at each end. All these -CHO groups will combine with any protein nitrogens with which they come into contact, so there is enormous potential for cross-linking, and that is just what happens (Fig. 4). There are also many left-over aldehyde groups (not bound to anything) that cannot be washed out of the tissue.

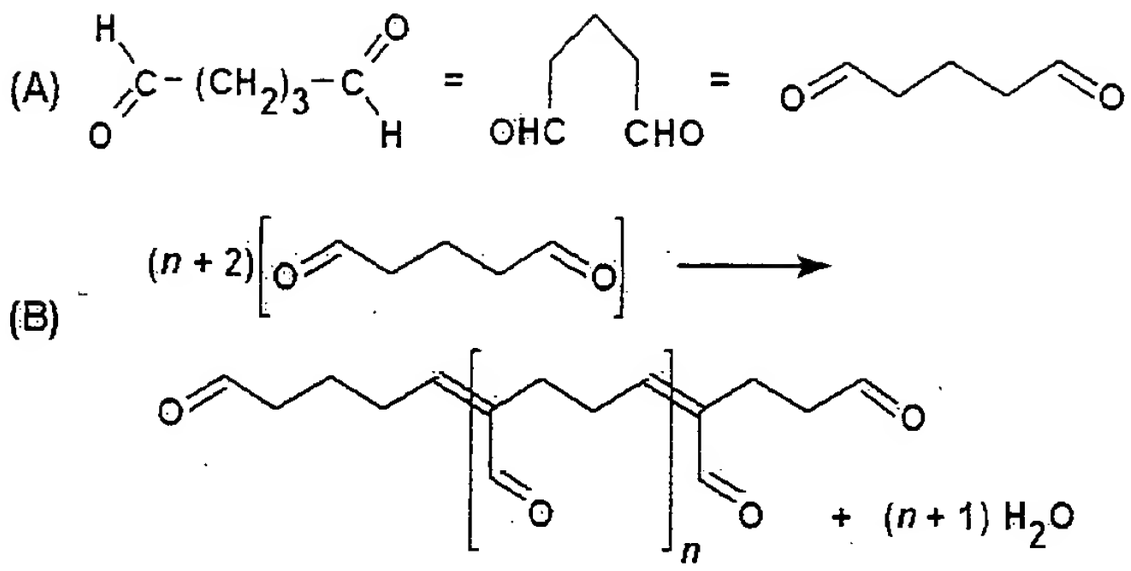

Fig. 3. **(A)** Three representations of a molecule of monomeric glutaraldehyde. **(B)** Polymerization reaction of glutaraldehyde, showing an aldehyde side-chain on each unit of the polymer.

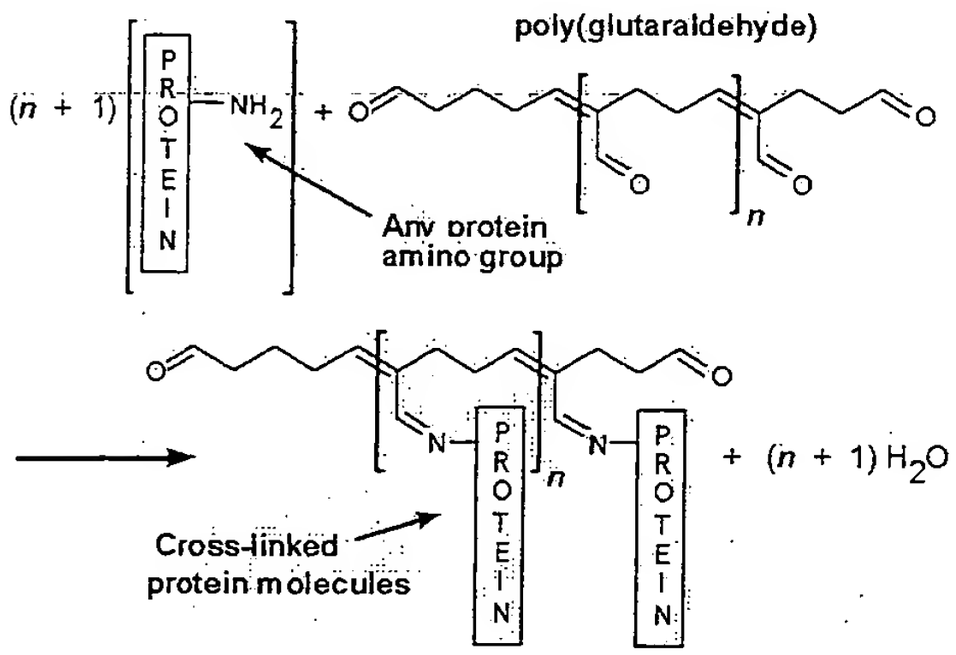

Fig. 4. Reaction of poly(glutaraldehyde) with amino groups of proteins.

Practical aspects of glutaraldehyde fixation

Five important points must be remembered when using glutaraldehyde as a fixative for light or electron microscopy.

1. If it's to be any use as a fixative, especially for electron microscopy, the glutaraldehyde solution must contain the monomer and low polymers (oligomers) with molecules small enough to penetrate the tissue fairly quickly. This means you must buy an "EM grade" glutaraldehyde (25% or 50% solution), not a cheaper "technical" grade. The cheaper stuff, which is for tanning leather, consists largely of polymer molecules too large to fit between the macromolecules of cells and other tissue components.

2. The chemical reaction of glutaraldehyde with protein is fast (minutes to hours), but the larger molecules, especially the oligomers, penetrate tissue slowly. A rat's brain left overnight in a buffered glutaraldehyde solution and sliced the next day shows a colour change and harder consistency to a depth of 2-3 mm. Objects fixed for a few hours in glutaraldehyde are no longer osmotically responsive (Paljarvi *et al.*, 1979).

3. The free aldehyde groups introduced by glutaraldehyde fixation cause various problems. These include non-specific binding of proteinaceous reagents, notably antibodies, and a direct-positive reaction with Schiff's reagent). The free aldehydes must be removed or blocked by appropriate histochemical procedures, as described in textbooks (Culling *et al.*, 1985; Kiernan, 1999, Ruzin, 1999), before attempting immunohistochemistry, lectin histochemistry, the Feulgen reaction of periodic acid-Schiff staining on glutaraldehyde-fixed material.

4. The thorough cross-linking of a glutaraldehyde-fixed specimen impedes the penetration of fairly large paraffin wax molecules. This makes for difficult cutting and peculiar differential shrinkage artifacts within the specimen. You can stain mitochondria nicely in cells surrounded by obviously abnormal spaces. This is an exaggeration of the inadequacy of formaldehyde and osmium tetroxide as fixatives to precede paraffin (Baker, 1958), and it also highlights the shortcomings of predominantly coagulant fixatives (AFA, Davidson's, Bouin etc), which preserve the micro-anatomy well but destroy or displace little things like organelles. Fortunately, plastic monomers penetrate glutaraldehyde-fixed tissue adequately. It has been shown that they do not enter every crevice (Horobin & Tomlinson, 1976), but there is enough support to allow the cutting of ultrathin sections for electron microscopy.

5. Immunohistochemistry, which requires as many intact amino acid side-chains as possible, is severely impaired by glutaraldehyde fixation. Nevertheless, clever people have generated antibodies to individual amino acids, that are glutaraldehyde-bound to protein. These allow the detection of soluble amino acid neurotransmitters such as glutamate, GABA and even glycine in presynaptic axon terminals in glutaraldehyde-perfused central nervous tissue (Hodgson *et al.*, 1985; Hepler *et al.*, 1988; Crooks & Kolb, 1992). Extensive cross-linking also results in the loss or severe reduction of most histochemically demonstrable enzymatic activities, though

several are retained after brief fixation (Sabatini *et al.*, 1962).

Mixtures containing formaldehyde and glutaraldehyde

The combination of formaldehyde with glutaraldehyde as a fixative for electron microscopy takes advantage of the rapid penetration of small HCHO molecules, which initiate the structural stabilization of the tissue. Rapid and thorough cross-linking is brought about by the more slowly penetrating glutaraldehyde oligomers. This mixture is associated with the name of Morris J. Karnovsky of Boston. It is an example of a great innovation that was published only in an unrefereed abstract (Karnovsky, 1965). His original mixture contained 4% glutaraldehyde, which was a higher concentration than many people wanted to use (Hayat, 1981). Designations like "half-strength Karnovsky" became common parlance in the 1960s and 1970s. Fixatives of this kind allowed the definitive descriptions of EM-level histology that were accomplished in the 5 or 6 years that followed the introduction of Karnovsky's fixative, and they are still routinely used.

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Last updated: September 2004 (to make the graphics readable with Internet Explorer as well as with Netscape)

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TEXTBOOK OF POLYMER SCIENCE

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Preface

"Dear Colleague, Leave the concept of large molecules well alone . . . there can be no such thing as a macromolecule."

It is said* that this advice was given to Hermann Staudinger just 45 years ago, after a major lecture devoted to his evidence in favor of the macromolecular concept. Today it seems almost impossible that this violent opposition to the idea of the existence of polymer molecules could have existed in relatively recent times. Now we take for granted not only the existence of macromolecules but their value to us in food, clothing, shelter, transportation, communication, most other aspects of modern technology, and last but far from least the muscles, sinews, genes, and chromosomes that constitute our bodies and intellect.

Even within the years since the first edition of *Textbook of Polymer Science* (1962) was written, the use of synthetic polymers has proliferated, as discussed in Chapter 7*E*. Not only has the annual production of plastics (for example) increased some 250% in the last eight years, but on a volume basis it has already exceeded that of copper and aluminum, and is expected to surpass the production of steel by the mid-1980's. One consequence of this widening use of polymeric materials is that a substantial if not major fraction of all chemists and chemical engineers, to say nothing of those in other disciplines, is employed in industry related in some way to polymers. Estimates vary, but this fraction appears to be one-third to one-half or higher.

Education in polymer science has not kept pace. By far the majority of colleges and universities in the United States have no courses in polymer

* Robert Olby, "The Macromolecular Concept and the Origins of Molecular Biology", *J. Chem. Educ.* 47, 168–174 (1970)

mechanism. Successive propagation steps involve, alternately, the addition of a monomer and the formation of a six-membered ring:

where X is one of a variety of groups. Five- and seven-membered rings form from the appropriate polymers with more difficulty, and some open-chain unsaturated groups occur in the polymer.

GENERAL REFERENCES

Alfrey 1952; Temin 1966.

D. Chemical Reactions of Polymers

The concept of the reactivity of functional groups being independent of molecular weight, used in developing the kinetics and statistics of stepwise polymerization (Chapter 8), applies to all functional groups regardless of their location on the polymer chain. As a result, polymers undergo chemical reactions much as do low-molecular-weight compounds provided that reactants can be supplied to the sites of reaction. To accomplish this, most polymer reactions of importance are carried out in solution. Many of these reactions are discussed elsewhere in this book, as indicated by cross reference.

A well-known sequence of polymer reactions is the conversion of poly(vinyl acetate) through poly(vinyl alcohol) to a poly(vinyl acetal) (Chapter 14*C*). In the latter reaction, as in the reaction of metals to remove chlorine from poly(vinyl chloride), functional groups react in pairs along the chain, with occasional groups isolated and incapable of reaction.

Polyesters are readily hydrolyzed unless low solubility or steric hindrance interferes. Thus, linear condensation polyesters and polyacrylates hydrolyze readily, whereas poly(ethylene terephthalate) and crosslinked alkyd and "polyester" resins are insoluble. Polymethacrylates are inert because of steric hindrance.

The nitration, sulfonation, and reduction of styrene polymers are widely used to produce ion-exchange resins (Chapter 14*A*). The acetylation,

nitration, and xanthation of cellulose (Chapter 15*C*) are important reactions, as are vulcanization and other reactions of natural and synthetic rubbers (Chapters 13*E–H* and 19).

In addition, unsaturated polymers can undergo such reactions as isomerization, cyclization, addition, epoxidation, and hydrogenation, while saturated polymeric hydrocarbons can be substituted on the main chain, the side chain, or the nucleus if aromatic. Terminally reactive polymers can produce block copolymers whereas branching reactions can lead to graft copolymers (Chapter 11*D*). Coupling reactions can promote increases in molecular weight, as in the polyurethanes (Chapter 15*D*), or crosslinking, as in a variety of thermosetting resins (Chapter 16). The class of oxidation-reduction or redox polymers is discussed by Cassidy (1965). Still other reactions of polymers are described in Sections *E* and *F* of this chapter.

GENERAL REFERENCES

Fettes 1964; Lenz 1967; Ravve 1967; Odian 1970.

E. Degradation of Polymers

In the classical chemical sense, the term degradation means breaking down of structure; in one usage relating to polymers (Grassie 1966) it is taken to mean any process leading to deterioration of properties; but here we take it to mean reduction of molecular weight. There are two general types of polymer degradation processes, corresponding roughly to the two types of polymerization, step-reaction and chain-reaction.

Random degradation is analogous to stepwise polymerization. Here chain rupture or scission occurs at random points along the chain, leaving fragments which are usually large compared to a monomer unit.

Chain depolymerization involves the successive release of monomer units from a chain end in a *depropagation* or *unzippering* reaction which is essentially the reverse of chain polymerization.

These two types may occur separately or in combination, may be initiated thermally or by ultraviolet light, oxygen, ozone, or other foreign agent, and may occur entirely at random or preferentially at chain ends or at other weak links in the chain.

It is possible to differentiate between the two processes in some cases by following the molecular weight of the residue as a function of the extent of reaction. Molecular weight drops rapidly as random degradation proceeds

but may remain constant in chain depolymerization, as whole molecules are reduced to monomer which escapes from the residual sample as a gas. Examination of the degradation products also differentiates between the two processes: the ultimate product of random degradation is likely to be a disperse mixture of fragments of molecular weight up to several hundred, whereas chain depolymerization yields large quantities of monomer.

Kinetics of random degradation

The kinetics and statistics of random degradation can be treated in exact analogy with the kinetics of linear stepwise polymerization. If p is defined, as before, as the extent of reaction, then $1 - p =$ (number of broken bonds)/(total number of bonds) is the extent of degradation. If the degradation is random, the number of bonds broken per unit time is constant as long as the total number of bonds present is large compared to the number broken. It follows that the number of chain ends increases linearly with time; hence, $1/\bar{x}_n$ increases linearly with time. The acid-catalyzed homogeneous degradation of cellulose is a random degradation of this type.

An example of random degradation initiated by attack at a "weak link" in a chain is the ozonolysis of the isobutylene-isoprene copolymer butyl rubber. Here the initial attack is at the double bonds of the isoprene residues.

Chain depolymerization

Chain depolymerization is a free radical process which is essentially the reverse of chain polymerization. The point of initial attack may be at the chain end or at a "weak link" which may arise from a chain defect, such as an initiator fragment or a peroxide or ether link arising from polymerization in the presence of oxygen. The slightly higher activity of a tertiary hydrogen atom may be enough to provide a site for the initiation of the degradation process.

Poly(methyl methacrylate) degrades thermally by this process. The yield of monomer is 100% of the weight of polymer lost over a large fraction of the reaction. Polystyrene shows an intermediate behavior: the depropagation reaction ceases before the chain is completely destroyed. In some cases, such as olefin-SO_2 copolymers, an equilibrium can easily be reached between propagation and depropagation.

Kinetics of chain depolymerization A general kinetic scheme has been formulated which appears to cover all types of depolymerization. It is based on the concept of inverse chain polymerization and includes the steps of initiation, depropagation, termination, and chain transfer. The important

feature of this scheme is the inclusion of the chain transfer step, for it can be shown that the kinetics of random degradation result if the kinetic chain length before transfer is just the breaking of one bond.

The transfer reaction probably occurs rapidly by the abstraction of a hydrogen atom from a polymer. The chain which was attacked is likely to split into a radical and one or more inactive fragments at the elevated temperatures where degradation is rapid. Evidence for the transfer reaction includes the observations that tertiary hydrogen atoms at branch points in polyethylene are preferentially attacked and that the degradation of poly(methyl methacrylate) which has been copolymerized with a little acrylonitrile is quite different from that of pure poly(methyl methacrylate) because of the activity of the α-hydrogens on the acrylonitrile units.

The two factors which appear to be important in determining the course of degradation are the reactivity of the depropagating radical and the availability of reactive hydrogen atoms for transfer. With the possible exception of styrene, where the radical is stabilized by resonance, all polymers containing α-hydrogens, such as polyacrylates and polyacrylonitrile, give poor yields of monomer.

Conversely, the methacrylates give high yields of monomer because of the active radical and the α-methyl group which blocks the possibility of chain transfer. Polytetrafluoroethylene gives high yields of monomer because the strong C-F bond is not easily broken to allow transfer.

The scheme does not apply to polymers such as poly(vinyl acetate) and poly(vinyl chloride), where degradation results from the removal of side groups rather than from chain scission.

Only two parameters beside the rate constants are important in the kinetic analysis. They are the transfer constant, σ, defined as

$$\sigma = \frac{\text{probability of transfer}}{\text{probability of initiation}} = \frac{k_{tr}[R\cdot]}{k_i} \tag{12-11}$$

and the kinetic chain length. This is not defined in the same way as in polymerization, but as the number-average number of monomers produced from one chain. The kinetic chain length is $(1/\varepsilon) - 1$, where

$$\frac{1}{\varepsilon} = \frac{\text{probability of (propagation + termination + transfer)}}{\text{probability of (termination + transfer)}} \tag{12-12}$$

The kinetic chain length is approximately measured by the ratio of weight of monomer to that of other volatile fragments when depolymerization is completed. In polyethylene, where almost no monomer is produced, $1/\varepsilon \simeq 1$. In polystyrene or polyisobutylene, $1/\varepsilon \simeq 4$; in poly(methyl methacrylate) $1/\varepsilon$ is at least as high as 200.

Jellinek (1966) has pointed out errors in some earlier treatments of depolymerization.

Degradation products Study of the products of thermal depolymerization in vacuum has shown that the chemical nature and relative amounts of these products are remarkably independent of the temperature and extent of the degradation reaction. Polystyrene, e.g., degraded to 40% styrene, 2.4% toluene, and other products having an average molecular weight of 264, at temperatures between 360° and 420°C, and extents of degradation from 4 to 100%. The amounts of monomer produced by various polymers are shown in Table 12-5.

TABLE 12-5. Per cent monomer resulting from thermal degradation

Polymer	Per Cent Monomer	
	Weight	Mole
Poly(methyl methacrylate)	100	100
Poly(α-methylstyrene)	100	100
Polyisobutylene	32	78
Polystyrene	42	65
Polybutadiene	14	57
SBR (butadiene-styrene copolymer)	12	52
Polyisoprene	11	44
Polyethylene	3	21

GENERAL REFERENCES

Madorsky 1964; Grassie 1966; Jellinek 1966, 1968; Pinner 1967; Ravve 1967; Reich 1967, 1968.

F. Radiation Chemistry of Polymers

The interaction of high-energy radiation with molecular substances involves the following sequence of events, regardless of the source of energy (photons, protons, electrons, neutrons, etc.). The molecules are first excited and ionized. Secondary electrons are emitted with relatively low speeds, and produce many more ions along their tracks. Within 10^{-12} sec or so, molecular rearrangements take place in the ions and excited molecules, accompanied by thermal deactivation or the dissociation of valence bonds. As far as

subsequent reactions are concerned, bond dissociation is the more important. It leads to the production of ions or radicals whose lifetimes depend on diffusion rates and may be weeks or months in solids at low temperatures.

The major effects in polymers arise from the dissociation of primary valence bonds into radicals, whose existence can be demonstrated by chemical methods or by EPR spectroscopy (Chapter 4*D*). The dissociations of C-C and C-H bonds lead to different results, degradation and crosslinking, which may occur simultaneously.

Degradation The major result of radiation is degradation by chain scission in 1,1-disubstituted polymers, such as poly(methyl methacrylate) and its derivatives, polyisobutylene, and poly(α-methylstyrene), and in polymers containing halogen, such as poly(vinyl chloride), poly(vinylidene chloride), and polytetrafluoroethylene. The tendency toward degradation is related to the absence of tertiary hydrogen atoms, a weaker than average C-C bond (low heat of polymerization), or unusually strong bonds (such as C-F) elsewhere in the molecule.

Degradation is, of course, evidenced by decrease in molecular weight, the weight-average molecular weight being inversely proportional to the amount of radiation received. In polymers with bulky side chains, such as poly(methyl methacrylate), extensive degradation of the side chains to gaseous products also occurs.

Crosslinking Crosslinking is the predominant reaction on the irradiation of polystyrene, polyethylene and other olefin polymers, polyacrylates and their derivatives, and natural and synthetic rubbers. It is accompanied by the formation of gel and ultimately by the insolubilization of the entire specimen. Radiation crosslinking has a beneficial effect on the mechanical properties of some polymers and is carried out commercially to produce polyethylene with enhanced form stability and resistance to flow at high temperatures (Chapter 13*D*).

Other reactions Radiation crosslinking is often accompanied by the formation of *trans*-vinylene unsaturation, both reactions resulting in the formation of hydrogen gas. If the irradiation is carried out in the presence of air, surface oxidation results. The resulting peroxides may be decomposed later in graft copolymerization (Chapter 11*D*).

GENERAL REFERENCES

Bovey 1958; Charlesby 1960; Chapiro 1966, 1969; Lenz 1967; Platzer 1967; Parkinson 1969; Tabata 1969.